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Cellular Fatty Acid Composition of Nine Pathovars of *Xanthomonas campestris*

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Abstract

Cellular fatty acids of 80 strains of *Xanthomonas campestris*, representing 9 different pathovars, were analyzed by gas-liquid chromatography and mass spectrometry. A total of 48 fatty acids were identified, the most important being the 16 : 0 (averaging at least 4.5 % of the total), the *cis*- and *trans*-9 16 : 1 (over 14.4 %), and the *iso* and *anteiso* 15 : 0 (over 30 %). Other major fatty acids (averaging over 1 % of total) were the saturated 14 : 0 and 15 : 0, the hydroxy-substituted *iso* 3-OH 11 : 0, 3-OH 12 : 0 and *iso* 3-OH 13 : 0, and the branch-chained *iso* 11 : 0, *iso* 16 : 0, *iso* 17 : 1, *iso* 17 : 0 and *anteiso* 17 : 0. Of 33 minor fatty acids detected and identified, only 7 have been previously reported in the xanthomonads. Significant differences in mean percentages of 5 major fatty acids and 4 (chemical) class totals were detected among pathovars, which statistically segregated into three groups by rank analysis. *X. campestris* pv. *dieffenbachiae* was in a group by itself; pvs. *campestris, citri* (pathotypes A and B), *manihotis, phaseoli, pruni* and *vesicatoria* were in a seond group, and pvs. *glycines, begonia* and *citri* (pathotype E) were in a third.

Zusammenfassung

Zelluläre Fettsäurezusammensetzung bei neun *Xanthomonas campestris*-Pathovaren

Die zellulären Fettsäuren von 80 *Xanthomonas campestris*-Stämmen, die 9 unterschiedliche Pathovaren vertreten, wurden durch HPLC und MS analysiert. Insgesamt wurden 48 Fettsäuren identifiziert, hauptsächlich die 16 : 0 (durchschnittlich mindestens 4,5 % der Gesamtanzahl), die *cis*-

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und *trans*-9 16 : 1 (mehr als 14,4 %), sowie die *iso* und *anteiso* 15 : 0 (mehr als 30 %) Säuren. Andere Festtsäuren, die häufig vertreten waren (im Durchschnitt mehr als 1 % der Gesamtanzahl), waren die saturierten 14 : 0 und 15 : 0, die Hydroxy-substituierten *iso* 3-OH 11 : 0, 3-OH 12 : 0 und *iso* 3-OH 13 : 0 sowie die verzweigt-kettigen *iso* 11 : 0, *iso* 16 : 0, *iso* 17 : 1, *iso* 17 : 0 und *anteiso* 17 : 0 Säuren. Von den gefundenen und identifizierten 33 Fettsäuren, die nicht so häufig vorkamen, ist es nur bei 7 schon beschrieben, daß sie in den Xanthomonaden vorkommen. Signifikante Unterschiede der Durchschnittsprozente bei 5 Hauptfettsäuren wurden festgestellt sowie 4 (chemische) Gesamtklassen zwischen den Pathovaren, die statistisch in drei Gruppen durch eine Rankanalyse differenziert werden konnten. *X. campestris* pv. *dieffenbachiae* war in einer Gruppe für sich, pvs. *campestris, citri* (Pathotypen A und B), *manihotis, phaseoli, pruni* und *vesicatoria* waren in einer zweiten Gruppe und pvs. *glycines, begonia* und *citri* (Pathotyp E) waren in der dritten Gruppe.

Xanthomonas campestris (Pammel) Dowson, an epiphytic or parasitic bacterium generally associated with plants, is subdivided into numerous pathovars based on host, and host ranges which are sometimes poorly defined (BRADBURY 1984). The need for improvements in phylogenetic classification of *X. campestris* pathovars is recognized and will require a better understanding of phenotypic and genetic as well as phytopathological characteristics (VAUTERIN *et al.* 1990). Among useful chemotaxonomic parameters is the composition of cellular fatty acids, presently used as a confirmatory tool in bacterial identification (LECHEVALIER 1977, SASSER 1990).

The general features of the fatty acid composition of *X. campestris* have been described in numerous reports (MATHYSHEVSKAYA 1981, MAAS *et al.* 1985, GITAITIS *et al.* 1987, STEAD 1989, GRAHAM *et al.* 1990, SASSER 1990, VAUTERIN *et al.* 1991). They include as major components (1 % or more of the total) the saturated straight-chained 16 : 0; *iso* and *anteiso*-branched 11-, 15- and 17-carbon chains; hydroxy-substituted *iso* 3-OH 11 : 0, 3-OH 12 : 0, and *iso* 3-OH 13 : 0; and the unsaturated 16 : 1 and *iso* 17 : 1. About 50 % of the total profile are branched-chains. Minor components include the saturated 10 : 0, 14 : 0, and 15 : 0; *iso*-branched 13 : 0 and 14 : 0; and the unsaturated 15 : 1 and 17 : 1 (VAUTERIN *et al.* 1991). STEAD (1989), in a preliminary study of 14 pathovars of *X. campestris*, and VAUTERIN *et al.* (1991) analyzing strains of pathovar *citri*, independently noticed different types of fatty acid profiles, differing primarily in percentages of *iso*-branched fatty acids.

None of the previous reports provided detailed analyses of fatty acid profiles of a range of pathovars of *X. campestris*. The analyses have been of limited detail containing less than 20 components, and in some cases based on statistics with no reference to the underlying data. The purpose of this report is to provide a basic description of the fatty acid composition of *X. campestris* using detailed analyses of over 40 identifiable components, and to verify if characteristic profiles exist for pathovars within the species.

Materials and Methods

Cultivation and maintenance of bacterial strains

This study was based on a collection of 4 to 13 strains of each of 8 pathovars of *X. campestris* and 25 strains of a ninth, *X. c.* pv. *citri*, which included representatives of pathotypes A, B and E (HARTUNG and CIVEROLO 1987) (Table 1). Bacteria were single-colony cloned three times (or once, if

received as pure cultures), and cultured on King's medium B agar (KB, Difco) for 24—30 hrs. at 25 °C prior to fatty acid analysis. For long term storage, strains were kept in trypticase soy broth +5 % DMSO (dimethyl-sulfoxide) at −90 °C.

Fatty acid analysis

Bacterial cells were saponified, esterified and analyzed for fatty acids by a method based on that of MOSS (1981) and modified by SASSER (1990). Approximately 400—500 mg (wet weight) of cells were saponified and esterified by mixing in 1 ml of 1.2 N NaOH in 50 % aqueous methanol, heated for 30 min in a boiling water bath, then combined with 0.5 ml 6 N HCl and 1 ml 12 % boron trichloride-methanol, and heated for 5 min at 85 °C. Methylated acids were extracted with 1 ml hexane-diethylether (1 : 1), washed with 3 ml of 0.3 N NaOH, and concentrated to approximately 20—30 µl under a stream of filtered, high-purity nitrogen gas. Two µl of the concentrate were injected into a Model 3700 Gas Chromatograph (Varian Associates, Sunnyvale, CA 94809) with a flame ionization detector and a 15 m × 0.25 mm capillary glass column coated with SPB-1 (Supelco, Inc.) as a non-polar stationary phase. Solvent blanks were periodically tested for impurities. Operating conditions were: helium carrier gas flow of 20 cc/min; injector temperature 230 °C; detector temperature 250 °C; initial column temperature 130 °C; final column temperature 230 °C; and temperature program rate of 4 °C/min. Generally, each strain was grown and tested one time. Strains of special interest (such as type strains) were tested more than once to establish limits of experimental variability.

Fatty acids between 8 and 20 carbons long were identified by co-chromatography with reference standards. Major fatty acid peaks were confirmed with a Finnegan 8230 HR mass spectrometer. Unsaturated fatty acids were confirmed chemically by hydrogenation of methyl esters for 15 min, by a method originally described by MOSS (1979). Upon hydrogenation, unsaturated acids disappeared and a corresponding increase was observed in the saturated, straight-chained analog. Hydroxy-substituted fatty acids were confirmed by trifluoroacetylation, also using the method of MOSS (1979). Upon acetylation, the retention times of the diacyl derivatives of these acids shifted when chromatographed. Methylated samples were tested for the presence of cyclopropane fatty acids by the method of BRIAN and GARDNER (1968). Known 17 : 0 and 19 : 0 cyclopropane fatty acids (Supelco, Inc., and Applied Science, State College, PA) were used as internal standards. Equivalent (carbon) chain length (ECL) was calculated for each peak, and provided further confirmation of identities by reference to published reports (GILLIAN and HOGG 1984, SASSER 1990). Fatty acids occurring at trace levels (less than 0.05 % of the total) were provisionally identified by matching ECLs or by co-chromatography with reference standards.

Data handling

Eluted fractions in each sample were integrated and quantified as percent of total peak area with a Model 4270 Integrator (Varian Associates). Data from the integrator was entered into an Apple III micro-computer programmed with an Omnis 3 database manager (Blythe Software Inc., San Mateo, CA 94403). Fatty acids were organized into chemical classes as described by ASSELINEAU (1962), and class totals were used to assist in comparative analyses of pathovars. Class A was the saturated, straight-chain, even-carbon fatty acids. Class B, saturated straight-chain, odd-carbon fatty acids; Class C, unsaturated acids; Class D, hydroxy-substituted acids; and Class E, branched chain acids. Class F, the cyclopropane fatty acids, were detected at trace levels only. Variation of percentages from sample means were calculated as sample standard deviations, and comparisons among means and rank analyses were by the methods of J. E. TUKEY as modified by G. SNEDECOR (1966). Unless otherwise mentioned, statistical differences were at the 99 % level of probability (P = 0.01).

Results

A total of 48 fatty acids from *X. campestris* were detected and identified. The most important components were the 16 : 0, averaging at least over 4.5 % of the total; the *cis*- and *trans*-9 16 : 1, averaging together over 14.4 % of total; and the

iso and *anteiso* 15 : 0, averaging over 30 % of total (Table 2). Other major fatty acids, each averaging over 1 % of total, were the saturated straight-chained 14 : 0 and 15 : 0; the hydroxy-substituted *iso* 3-OH 11 : 0, 3-OH 12 : 0, and *iso* 3-OH 13 : 0; and the branched-chained *iso* 11 : 0, *iso* 16 : 0, *iso* 17 : 0 and *anteiso* 17 : 0. Of the 33 minor fatty acids detected, 7 have been previously reported in the xanthomonads (GITAITIS *et al.* 1987, VAUTERIN *et al.* 1991). The branched chain (Class E) and the unsaturated (Class C) fatty acids comprised the largest chemical classes, averaging over 44 % and 16 % of the total, respectively (Tables 2 and 3).

Table 1

Strains of *Xanthomonas campestris* pathovars used in this study

Pathovar	Strain designations	Source or reference[a]
X. c. pv. *begonia*	XB1, 2	E. Civerolo *et al.* (1982)
	ATCC 8718, 11725	ATCC
X. c. pv. *campestris*	CJ-92, -93, TJ71, PF83	C. Liao, original isolations
	XCC-42, XCC-43	UWCC via W. Fett
	XC-1, -2, -4, -5	J. Wells, original isolations
	ATCC 33913	ATCC
X. c. pv. *citri* (A)	XC-118 (NCPPB 2056)	NCPPB
	M-174, XC-143, XC-59,	J. Hartung and E. Civerolo (1987)
	XC-62, -63, -97, -98, -99	J. Hartung and E. Civerolo (1987)
X. c. pv. *citri* (B)	XC-64, -69	J. Hartung and E. Civerolo (1987)
X. c. pv. *citri* (E)[b]	F-6, -16, -17, -19, -29, -100	J. Hartung and E. Civerolo (1990)
	-231, -254, -258, -294, -300	J. Hartung and E. Civerolo (1990)
	ATCC 49120	ATCC
X. c. pv. *dieffenbachiae*	M-8, -11, -12, -18, -19, -24	K. Pohronezny, original isolations
	ATCC 23379	ATCC
X. c. pv. *glycines*	1135, 1717	NCPPB via W. Fett
	ATCC 43912	ATCC
	XP144, 1136	W. Fett (FETT *et al.* 1987)
	S-9-8[c], 1136[c]	W. Fett (FETT *et al.* 1987)
X. c. pv. *manihotis*	XM-1, -2, -4, -6	E. Civerolo (CIVEROLO *et al.* 1982)
	ATCC 23380	ATCC
X. c. pv. *phaseoli*	CL11	C. Liao, original isolations
	XPh1 (NCPPB 1811)	NCPPB
	753F, 885F	A. Gould via G. O'Keefe

Table 1 (Continued)

Pathovar	Strain designations	Source or reference[a]
	BSB	R. Gitaitis via W. Fett
	ATCC 9563	ATCC
	Xcp 27	A. Saettler via W. Fett
X. c. pv. *pruni*	NCCPB 1196	NCPPB
	XP-1, -3, -4, -28, -31	E. Civerolo (CIVEROLO *et al.* 1982)
	ATCC 19316	ATCC
X. c. pv. *vesicatoria*	CL13	C. Liao, original isolations
	XV-1, -2	E. Civerolo (CIVEROLO *et al.* 1982)
	ATCC 11551	ATCC
	RG	R. Gitaitis via G. O'Keefe
	T9196, P9153, P9157, T6890	G. O'Keefe, original isolations

[a] ATCC = American Type Culture Collection, Rockville, MD; UWCC = University of Wisconsin Culture Collection. U. of Wisc., Madison, WI; NCPBB = National Collection of Plant Pathogenic Bacteria, Harpenden, England; K. Pohronezny, U. of Florida, EREC, Belle Glade, FL; C. Liao and W. Fett, USDA, 600 E. Mermaid La., Philadelphia, PA; A. Gould, Univ. of Florida, Monticello, FL; G. O'Keefe, U. of Georgia, Coastal Plain Expt. Sta., Tifton, GA.

[b] All E strains belong to the "moderately aggressive" type 4 of the citrus bacterial spots bacterium (HARTUNG and CIVEROLO 1990).

[c] Avirulent isolates.

Mean percentages of 4 of the 6 classes and 5 of the 15 major fatty acids were significantly different in some pathovars of *X. campestris.* Pathovars *dieffenbachiae, campestris* and *glycines* typified these differences. Percentages for pv. *dieffenbachiae* differed from *campestris* and *glycines* in all 9 of the above factors except the *iso*-15 : 0 (Table 2). In the case of *iso*-15 : 0, the mean percentage for pv. *campestris* (28.02 %) was different from both pvs. *dieffenbachiae* and *glycines* (12.28 and 18.51 %, respectively).

Two other parameters derived from fatty acid percentages were equally useful in differentiating the groups: the ratios of Class E/C, and of the branched 15 : 0 divided by the *cis* and *trans* 16 : 1 (Table 3). Ratios for pv. *dieffenbachiae* and *glycines* were significantly different from each other and those for pv. *campestris* were in the intermediate range.

There were also some significant differences in fatty acid composition in the pathotypes of *X. campestris* pv. *citri.* Mean percentages for types A and B were similar (and thus combined), but type E was distinct in all factors that differentiated pathovars. While the composition of pv. *citri* types A and B was similar to the group typified by pv. *campestris,* the composition of type E was similar to the group of pv. *glycines* (Table 3).

Table 2

Mean percentages of total fatty acids in strains of *Xanthomonas campestris* pvs. *dieffenbachiae*, *campestris*, and *glycines* grown for 1 day on Kings's B agar medium at 25 °C

Fatty acids[w]		*X. campestris* pathovars[x]		
Chemical class	ECL[y]	*dieffenbachiae*	*campestris*	*glycines*
Class A:				
8:0	8.0	0.06[z]	1.09	0.15
10:0	10.0	0.68	0.60	1.20
12:0	12.0	0.31	0.05	0.01
14:0	14.0	1.32	1.49	3.13
16:0	16.0	4.46 a	4.82 a	13.12 b
18:0	18.0	0.09	0.10	0.17
20:0	20.0	0.01	0.12	0.04
Total : z		6.92 a	8.27 ab	17.81 b
S.D.		±0.79	±2.29	±2.00
Class B:				
9:0	9.0	0	0.05	0.02
11:0	11.0	0.03	0.08	0.10
13:0	13.0	0.20	0.10	0.14
15:0	15.0	0.32 a	1.70 ab	2.26 b
17:0	17.0	0.06	0.16	0.37
19:0	19.0	0.09	0.03	0.02
Total : z		0.69 a	2.13 b	2.90 b
S.D.		±0.17	±0.61	±0.89
Class C:				
12:1-*cis* 5	11.79	0	0.02	0
14:1-*cis* 9	13.84	0.98	0.06	0.01
15:1	14.85	0.22	0.54	0.43
16:1-*cis* 9	15.74	1.08	2.59	2.46
16:1-*trans* 9	15.82	13.30 a	15.28 ab	21.93 b
17:1-*cis* 9	16.81	0.10	0.55	0.10
17:1-*cis* 11	16.85	0.03	0.15	0.16
18:1-*cis* 11	17.73	0.29	0.56	0.61
18:1-*trans* 11	17.81	0.31	0.18	0.29
20:4-*cis* 5	19.20	0	0.02	0
Total : z		16.32 a	19.94 ab	25.98 b
S.D.		±3.53	±3.06	±2.57
Class D:				
2-OH 10:0	11.15	0.09	0.06	0.06
3-OH 10:0	11.43	0.17	0.07	0.24
iso 3-OH 11:0	12.10	1.08	1.62	1.79

Ranking of the 9 *X. campestris* pathovars (including the 2 pathotype subgroupings of pv. *citri*) by each of the differentiating factors and the 2 ratios, and then averaging rank orders, suggested that *X. campestris* pv. *dieffenbachiae* was in a statistical group by itself with a mean rank of 1.3 (Table 3). *X. campestris* segregated into a second group that included pvs. *citri* (pathotypes A and B),

Table 2 (Continued)

Fatty acids[w]		*X. campestris* pathovars[x]		
Chemical class	ECL[y]	*dieffenbachiae*	*campestris*	*glycines*
3-OH 11:0	12.42	0.03[z]	0.10	0.27
2-OH 12:0	13.16	0.10	0.05	0.06
3-OH 12:0	13.45	1.16	1.01	2.10
iso 3-OH 13:0	14.10	2.23	2.32	2.55
3-OH 14:0	15.49	0.03	0	0
2-OH 16:0	17.19	0.02	0	0
3-OH 16:0	17.50	0	0.02	0
iso 3-OH 17:0	18.14	0	0.01	0
3-OH 17:0	18.52	0.02	0.13	0.14
Total : z		4.93 a	5.38 a	7.21 a
S.D.		±0.72	±1.39	±1.44
Class E:				
iso-11:0	10.57	1.87	3.66	3.36
anteiso-12:0	11.71	0.45	0.88	0.94
iso-13:0	12.62	0.38	0.35	0.19
iso-14:0	13.64	0.33	0.53	0.21
iso-15:1	14.43	0.31	0.83	0.21
iso-15:0	14.63	12.28 a	28.02 b	18.51 a
anteiso-15:0	14.72	41.61 a	17.62 b	12.01 b
iso-16:0	15.65	2.04	1.29	0.86
iso-17:1	16.42	3.10	4.31	2.12
iso-17:0	16.64	3.14	3.95	3.90
anteiso-17:0	16.73	2.87	1.16	2.07
Total : z		68.36 a	62.61 a	44.40 b
S.D.		±3.89	±5.79	±3.61
Class F:				
D-17:0	16.85	0.01	0.01	0.01
D-19:0	18.86	0.01	0.04	0
Total : z		0.02 a	0.05 a	0.01
S.D.		±0.05	±0.05	±0.03
Unidentified:		2.76	1.62	1.69

[w] Classes: A = saturated, even-carbon straight chains; B = saturated, odd-carbon straight chains; C = unsaturated; D = hydroxy-substituted; E = branched chains; F = cyclopropane acids. ECL = equivalent (carbon) chain lengths as calculated from chromatographs.

[x] Mean percentages of 7 strains of *X. campestris* pv. *dieffenbachiae*, 10 strains of pv. *campestris* and 7 strains of pv. *glycines* (Table 1).

[y] ECL = equivalent (carbon) chain length.

[z] Mean percentage of total ± standard deviation (S.D.). Means in same row not followed by the same letter are significantly different (P = 0.01).

manihotis, phaseoli, pruni and *vesicatoria* — all having mean ranks of 4.0 to 6.3. Pathovars *begonia, glycines* and pathotype E of pv. *citri* were in a third group with means ranks of 8.0 to 9.4.

The ratio of *iso*-15 : 0 to *anteiso*-15 : 0, used by STEAD (1989) to differentiate pathovars, and by SASSER (1991) as a test for *Xanthomonas*, fell within the range of 1 to 10 for strains in all pathovars exept pv. *dieffenbachiae* (Table 3).

Table 3

Percentages (of total) of fatty acid classes of nine pathovars of *Xanthomonas campestris*, grouped by rank analysis of differentiating factors

Fatty acid classes and other differentiating factors[x]	Pathovars of *X. campestris*[w] (and number of strains)								
	Group A	Group B					Group C		
	dieffenbachiae (7)	*citri* A and B (11)	*campestris* (11)	*manihotis* (5)	*phaseoli* (7)	*pruni* (7)	*vesicatoria* (9)	*glycines* (7)	*begonia* (4)	*citri* E (12)
Class A[v]	6.92 a	10.37 abc	8.27 ab	11.34 bcd	9.72 ab	12.18 d	7.23 ab	17.81 e	12.69 d	16.69 e
S.D.	±0.79	±1.67	±2.29	±4.09	±1.44	±3.32	±1.75	±2.00	±5.55	±2.79
Class B	0.69 a	3.28 bcd	2.13 bcd	2.54 bcd	1.67 abc	3.04 d	1.50 ab	2.90 bcd	2.48 bcd	2.51 bcd
S.D.	±0.17	±1.21	±0.61	±1.40	±0.77	±1.15	±0.76	±0.89	±0.38	±0.81
Class C	16.32 a	18.15 ab	19.95 abc	22.16 bc	19.54 ab	18.02 a	24.28 cd	25.98 d	26.17 d	24.74 cd
S.D.	±3.53	±3.07	±3.07	±1.65	±3.22	±1.59	±2.52	±2.57	±1.05	±2.42
Class D	4.93 a	4.14 a	5.38	4.85 a	5.24 a	4.05 a	4.65 a	7.21 a	6.15 a	4.86 a
S.D.	±0.72	±1.66	±1.39	±1.02	±0.90	±2.01	±1.34	±1.44	±0.39	±1.14
Class E	68.36 a	62.99 ab	62.61 ab	58.28 b	61.55 ab	60.65 ab	60.95 ab	44.40 d	51.37 c	49.18 c
S.D.	±3.89	±3.54	±5.79	±5.50	±2.55	±4.59	±2.68	±3.61	±6.75	±2.80
Class F	0.03 a	0.05 a	0.05 a	0.01 a	0.03 a	0.01 a	0.02 a	0.01 a	0.05 a	0.41 a
S.D.	±0.03	±0.07	±0.04	±0.03	±0.04	±0.02	±0.03	±0.05	±0.05	±0.15
15:0	0.32 a	1.94 b	1.70 b	2.28 b	1.31 b	1.90 b	1.20 b	2.26 b	2.06 b	2.53 b
S.D.	±0.08	±0.52	±0.80	±0.59	±0.61	±0.77	±0.75	±0.65	±0.61	±0.78
16:0	4.46 a	6.18 ab	4.82 a	6.02 a	5.07 a	8.52 b	4.56 a	13.12 c	8.75 b	11.63 c
S.D.	±0.76	±1.75	±1.84	±2.15	±1.41	±2.27	±1.35	±1.97	±2.83	±2.32
16:1-<u>cis</u> + <u>trans</u> 9	14.38 a	16.27 a	17.87 ab	19.55 ab	18.44 a	17.38 a	22.18 bc	24.39 c	23.69 c	23.38 c
S.D.	±3.34	±2.82	±3.21	±1.92	±2.99	±1.53	±2.67	±2.53	±1.04	±2.20
<u>iso</u> + <u>anteiso</u>-15:0	53.90 a	42.75 bcd	45.64 bc	42.00 cd	47.06 bc	48.05 ab	44.59 bc	30.51 f	38.05 de	35.41 e
S.D.	±5.28	±5.28	±2.73	±4.78	±1.48	±4.29	±1.48	±2.29	±4.47	±3.21

Table 3 (Continued)

Fatty acid classes and other differentiating factors[x]	Pathovars of *X. campestris*[w] (and number of strains)									
	Group A	Group B						Group C		
	dieffen-bachiae (7)	*citri* A and B (11)	*campes-tris* (11)	*mani-hotis* (5)	*phase-oli* (7)	*pruni* (7)	*vesica-toria* (9)	*glycines* (7)	*begonia* (4)	*citri* E (12)
Ratios:[y]										
E/C	3.82 a	3.68 a	2.99 abc	2.69 bc	3.24 ab	3.48 ab	2.55 bcd	1.72 d	1.99 cd	1.98 cd
S.D.	±0.90	±0.70	±0.76	±0.39	±0.55	±0.57	±0.34	±0.26	±0.33	±0.30
iso + anteiso-15:0/16:1	3.43 a	2.73 ab	2.43 b	2.18 bc	2.60 b	2.80 b	2.04 bc	1.26 d	1.61 cd	1.52 cd
S.D.	±0.98	±0.73	±0.58	±0.47	±0.39	±0.50	±0.28	±0.19	±0.25	±0.28
iso-15:0/anteiso-15:0	0.29	3.26	1.59	2.04	1.70	1.38	1.57	1.54	3.00	2.40
Mean rank[z]	1.3 a	4.0 b	4.6 b	6.3 b	4.1 b	4.3 b	4.6 b	9.4 c	8.4 c	8.0 c

[w] See Table 1 for listing of strains. Cells grown for 1 day at 25 °C on King's B agar medium.

[x] Classes: A = saturated, even-carbon straight chains; B = saturated, odd-carbon straight chains; C = unsaturated; D = hydroxy-substituted; E = branched chains; F = cyclopropane acids.

[y] Mean percentages of total fatty acids ± sample standard deviation (S.D.). Means in each row not followed by the same letter are significantly different (P = 0.01). Mean ratios calculated from data of individual strains.

[z] Means based on rank analysis of all factors except Classes D and F and the ratio i-15:0/a-15:0.

Discussion

This study is intended to provide a detailed analysis of the fatty acid composition of a limited number of pathovars of *X. campestris*. It is not a taxonomic study. Nevertheless, it is interesting that the variation we detected in fatty acid composition of the pathovars, and their three distinct groupings, generally corresponded to findings of other researchers concerned with the taxonomy of *X. campestris* pathovars. VAUTERIN *et al.* (1991) noted several types of fatty acid profiles among strains of *X. campestris* pv. *citri*. Strains of pv. *citri* type A (and some of B) fit one profile, and those of type E fit another — corresponding to the second and third groups described herein. Recent studies of restriction fragment length polymorphisms (RFLP) of strains of pv. *citri* (HARTUNG and CIVEROLO 1989, GRAHAM *et al.* 1990) also noted a difference between the pathotypes.

With regard to the broader comparisons of pathovars of *X. campestris*, our data agrees with STEAD (1989) that fatty acid analyses can differentiate among some pathovars. Our results are, however, somewhat at variance with conclusions of VAUTERIN *et al.* (1990) who reported four fatty acid profile groups in a study of 29 cereal pathovars, and found that pv. *phaseoli* fit in a group that included pv. *glycines* rather than pv. *campestris*. But, since significant strain heterogeneity and no actual data was reported, it is difficult to reconcile the discrepancies. DNA hybridization studies have not provided strong support for natural grouping among pathovars studied (HILDEBRAND *et al.* 1990, VAUTERIN *et al.* 1990).

STEAD (1989) reported 40 fatty acids detected in *Xanthomonas*. We detected 48, but several of them were at trace levels and were not present in all strains; among them were the cyclopropane fatty acids. A persistent question in these analyses has been the likelihood of some fatty acids being artifacts of the saponification and methylation procedures rather than intrinsically occurring in the cell. Suspect might be the *trans* isomers of the 16 : 1 fatty acid, a form not generally found in nature. Nevertheless, these methyl ester components appear as direct or indirect products of the cells and are of value in the analysis.

The fatty acid groupings suggested in this report should be taken as provisional, subject to confirmation with larger collections of strains of each pathovar. However, if variability among strains (indicated by standard deviation values) are low, as was the case in this report, significant differences can be statistically detected with smaller sample numbers. For example, although data for only 12 strains of *X. campestris* pv. *citri* E was included in this report, mean values for all fatty acids and class totals were the same statistically for 5 randomly selected strains as for the 12, or for 31 which was the total number in our database.

Among the interesting results from this study, was confirmation of conclusions of GABRIEL *et al.* (1989) and VAUTERIN *et al.* (1991) that pathotype E of *X. campestris* pv. citri was biochemically distinct from pathotype A and B — and C and D, based on a limited sampling (unpublished data). Finally, it should be stated that the fatty acid profile of pv. *dieffenbachiae* was distinct in this study

only in the sense that we may not have included other pathovars of similar profile types. Dendrograms based on similarity coefficients of RFLP and DNA probes indicated pv. *dieffenbachiae* was related to pvs. *alfalfae* and *cyamopsidis* (GABRIEL *et al.* 1989), pathovars not included in this study.

Literature

ASSELINEAU, J., 1962: The Bacterial Lipids. Holden-Day Inc. Publishers, San Francisco, CA, USA, 372 pp.

BRIAN, L. B., and E. W. GARDNER, 1968: A simple procedure for detecting the presence of cyclopropane fatty acids in bacterial lipids. Appl. Microbiol. **16,** 549—552.

BRADBURY, J. F., 1984: *Xanthomonas.* In: KRIEG, N. R., and J. G. HOLT (eds), Bergey's Manual of Systematic Bacteriology, Vol. I, pp. 198—210. Williams & Wilkins Co., Baltimore, MD.

CIVEROLO, E. L., M. SASSER, C. HELKIE, and B. BURBAGE, 1982: Selective medium for *Xanthomonas campestris* pv. *pruni.* Plant Disease **66,** 39—43.

FETT, W. F., M. F. DUNN, G. R. MAHER, and B. E. MALEEFF, 1987: Bacteriocins and temperate phage of *Xanthomonas campestris* pv. *glycines.* Curr. Microbiol. **16,** 137—144.

GABRIEL, D. W., M. T. KINGSLEY, J. E. HUNTER, and T. GOTTWALD, 1989: Reinstatement of *Xanthomonas citri* (ex Hasse) and *X. phaseoli* (ex Smith) to species and reclassification of all *X. campestris* pv. *citri* strains. Int. J. System. Bacteriol. **39,** 14—22.

GILLAN, F. T., and R. W. HOGG, 1984: A method for the estimation of bacterial biomass and community structure in mangrove-associated sediments. J. Microbiol. Methods **2,** 275—293.

GITAITIS, R. D., M. J. SASSER, R. W. BEAVER, T. B. MCINNES, and R. E. STALL, 1987: Pectolytic Xanthomonads in mixed infections with *Pseudomonas syringae* pv. *syringae, P. syringae* pv. *tomato,* and *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper transplants. Phytopathology **77,** 611—615.

GRAHAM, J. H., J. S. HARTUNG, R. E. STALL, and A. R. CHASE, 1990: Pathological, restriction-fragment length polymorphism, and fatty acid profile relationships between *Xanthomonas campestris* from citrus and noncitrus hosts. Phytopathology **80,** 829—836.

HARTUNG, J. S., and E. L. CIVEROLO, 1987: Genomic fingerprints of *Xanthomonas campestris* pv. *citri* strains from Asia, South America, and Florida. Phytopathology **77,** 282—285.

— —, and — —, 1989: Restriction fragment length polymorphisms distinguish *Xanthomonas campestris* strains isolated from Florida citrus nurseries. Phytopathology **79,** 793—799.

— —, and — —, 1990: Variation among strains of *Xanthomonas campestris* causing citrus bacterial spot. Plant Dis. **75,** 622—626.

HILDEBRAND, D. C., N. J. PALLERONI, and M. N. SCHROTH, 1990: Deoxyribonucleic acid relatedness of 24 *Xanthomonas* strains representing 23 *Xanthomonas campestris* pathovars and *Xanthomonas fragariae.* Int. J. System. Bacteriol. **40.**

LECHEVALIER, M. P., 1977: Lipids in bacterial taxonomy — a taxonomist's view. CRC Critical Reviews in Microbiology **5,** 109—210.

MAAS, J. L., M. M. FINNEY, E. L. CIVEROLO, and M. SASSER, 1985: Association of an unusual strain of *Xanthomonas campestris* with apple. Phytopathology **75,** 438—445.

MATYSHEVSKAYA, M. S., 1981: Composition of fatty acids of the genus *Xanthomonas* bacteria (in Russian). Mikrobiol. Zh. **43,** 448—453.

MOSS, C. W., 1979: Analysis of cellular fatty acids of bacteria by gas-liquid chromatography. In: JONES, G. L., and G. A. HERBERT (eds), "Legionnaires", the Disease, Bacteria, and Methodology, pp. 117—122. U.S. Dept. Health, Education and Welfare, Atlanta, GA, USA.

— —, 1981: Gas-liquid chromatography as an analytical tool in microbiology. J. Chromatogr. (Amsterdam) **203,** 337—347.

SASSER, M., 1990: Identification of bacteria through fatty acid analysis. In: KLEMENT, F., K. RUDOLF, and D. C. SANDS (eds), Methods in Phytobacteriology, pp. 199—204. Akademiai Kiado, Budapest.

SNEDECOR, G. W., 1966: Statistical Methods. 5th Edition. Iowa State Univ. Press, Ames, IA, USA.

STEAD, D. E., 1989: Grouping of *Xanthomonas campestris* pathovars of cereals and grasses by fatty acid profiling. OEPP (Organ. Eur. Mediterr. Prot. Plant)/EPPO (Eur. Mediterr. Plant Prot. Organ.) Bull. **19**, 57—68.

VAUTERIN, L., J. SWINGS, K. KERSTERS, M. GILLIS, T. W. MEW, M. N. SCHROTH, N. J. PALLERONI, D. C. HILDEBRAND, D. E. STEAD, E. L. CIVEROLO, A. C. HAYWARD, H. MARAITE, R. E. STALL, A. K. VIDAVER, and J. F. BRADBURY, 1990: Towards an improved taxonomy of *Xanthomonas*. Int. J. System. Bacteriol. **40**, 312—316.

——, P. YANG, B. HOSTE, M. VANCANNEYT, E. CIVEROLO, J. SWINGS, and K. KERSTERS, 1991: Differentiation of *Xanthomonas campestris* pv. *citri* strains by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins, fatty acid analysis, and DNA-DNA hybridization. Int. J. Syst. Bacteriol. **41**, 9—16.